UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/632,794 | 08/04/2003 | Huai-Jen Tsai | 8961-000004/US | 5554 |

30596 7590 03/03/2006

HARNESS, DICKEY & PIERCE, P.L.C.
P.O.BOX 8910
RESTON, VA 20195

| EXAMINER | |
|---|---|
| BERTOGLIO, VALARIE E | |
| **ART UNIT** | **PAPER NUMBER** |
| 1632 | |

DATE MAILED: 03/03/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/632,794 | TSAI, HUAI-JEN | |
| | Examiner | Art Unit | |
| | Valarie Bertoglio | 1632 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *17 January 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-9* is/are pending in the application.

4a) Of the above claim(s) *1 and 2* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3-9* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *04 August 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *12/31/2003*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

Applicant's election without traverse of Group II, claims 3-9, in the reply filed on 01/17/2006 is acknowledged.

### *Election/Restrictions*

Claims 1 and 2 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 01/17/2006. Claims 1-9 are pending and claims 3-9 are under examination in the instant office action.

### *Specification*

The disclosure is objected to because of the following informalities: The pages are not numbered consecutively [see 37 CFR 1.52(b)(5)]. Appropriate correction is required.

### *Drawings*

Color photographs and color drawings are not accepted unless a petition filed under 37 CFR 1.84(a)(2) is granted. Any such petition must be accompanied by the appropriate fee set forth in 37 CFR 1.17(h), three sets of color drawings or color photographs, as appropriate, and, unless already present, an amendment to include the following language as the first paragraph of the brief description of the drawings section of the specification:

> The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Color photographs will be accepted if the conditions for accepting color drawings and black and white photographs have been satisfied. See 37 CFR 1.84(b)(2).

***Claim Objections***

Claim 3 is objected to because of the following informalities: The language of claim 3 is awkward. Modifiers such as "a" or "an" are lacking. For example, step (a) should read "an ITR" and "a CMV promoter". Step (b) should read "an α-actin gene promoter". Furthermore, the phrase "from upstream to downstream" is awkward. While it is clear the phrase is limiting the placement of each element of the plasmid, it would be more clear to require the plasmid comprise particular elements in order. Appropriate correction is required.

Claim 3 is objected to because of the following informalities: the parenthetical phrase in step (b) is misleading. While it is assumed Applicant is referring to the characteristic activity of the α-actin gene promoter as taught by the specification, the phrase is referring to actin gene expression as part of the activity. The actin gene is not expressed from the claimed plasmid because only the promoter, and the gene, is included in the plasmid. The parenthetical expression should be clarified or removed from the claim. Appropriate correction is required.

Claims 3,5 and 6 are objected to because of the following informalities: the claims require use of a fluorescent gene in a plasmid construct. Genes are not fluorescent, but the protein products can be. As such, claim 3, for example, should read "a gene encoding a fluorescent gene product" rather than "fluorescent gene". Appropriate correction is required.

Claim 4 is objected to because of the following informalities: Claim 4 contains drawings, which are not permitted to be part of the specification, including the claims [see MPEP 608.01 (VI) and 37 CFR 1.58(a)]. Appropriate correction is required.

***Claim Rejections - 35 USC § 112-1st paragraph***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 4 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claim 4 appears to require specific plasmids recited by the specific names p-αDsRedITR (8.0 kb) and p-αEGFPITR (8.1 kb). Since the plasmids appear to be essential to the claimed invention it must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. If the plasmid is not so obtainable or available, the requirements of 35 USC 112 may be satisfied by a deposit of the plasmid. 37 CFR 1.802. The specification does not disclose a repeatable process to obtain the plasmid and it is not apparent if the plasmid is readily available to the public. Thus, a deposit is required for enablement purpose. If the deposit is made under the terms of the Budapest Treaty, then an affidavit or declaration by applicants, or a statement by an attorney of record over his or her signature and registration

number, stating that the specific plasmids have been deposited under the Budapest Treaty and that the plasmids will be irrevocably and without restriction or condition released to the public upon the issuance of a patent, would satisfy the deposit requirement made herein (37 CFR 1.808).

If the deposit has not been made under the Budapest Treaty, then in order to certify that the deposit meets the criteria set forth in 37 CFR 1.808, applicants may provide assurance of compliance by an affidavit or declaration, or by a statement by an attorney of record over his or her signature and registration number, showing that

(a) during the pendency of this application, access to the invention will be afforded to the Commissioner upon request;

(b) all restrictions upon availability to the public will be irrevocably removed upon granting of the patent;

(c) the deposit will be maintained in a public depository for a period of 30 years or 5 years after the last request or for the effective life of the patent, whichever is longer;

(d) a viability statement in accordance with the provisions of 37 CFR 1.807; and

(e) the deposit will be replaced if it should ever become inviable.

As required under 37 CFR 1.809(d), the specification shall contain: (1) the accession number for the deposit; (2) the date of deposit; (3) a description of the deposited biological material sufficient to identify it and to permit its examination; and (4) the name and address of the depository.

In addition, the following issues must also be addressed for claim 4.

Claim 4 depends from claim 3, which requires selecting eggs with fluorescence. However, expression of a transgene will not occur in eggs because transcription does not occur until later in embryogenesis, after the midblastula transition (see below). Therefore, unless the DNA itself was fluorescent, fluorescence could not be determined in the fertilized egg. Accordingly, the specification taught detecting fluorescence after 24 hours [paragraph 0024]. The specification does not teach fluorescence in fertilized eggs.

Furthermore, claim 3 requires cultivating eggs to produce golden zebrafish with systemic fluorescence. The α-actin promoter drives expression in skeletal muscle and does not drive expression in other organ systems. The term "systemic fluorescence" is so broad as to encompass fluorescence in systems other than skeletal muscle that are not supported or enabled by the specification. Accordingly, the claims should be limited to skeletal muscle expression.

Claims 3 and 5-9 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of producing a transgenic golden zebrafish with systemic skeletal muscle fluorescence comprising constructing a plasmid including, in order, a first ITR, a zebrafish α-actin promoter operably linked to a gene encoding a fluorescent protein, an SV40 polyA and a second ITR, linearizing said plasmid, injecting said linearized plasmid into fertilized eggs of golden zebrafish, selecting fluorescent embryos and cultivating said fluorescent embryos to produce golden zebrafish with systemic skeletal muscle fluorescence does not reasonably provide enablement for use of the claimed construct wherein the promoter and reporter gene are not operably linked, selecting eggs with fluorescence or producing zebrafish

with systemic fluorescence in any tissue other than the skeletal muscle. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and or use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(a)). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (*Wands*, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (*Wands*, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case are discussed below.

The claims are drawn to a method of making a zebrafish exhibiting systemic fluorescence and the resultant fish. Claim 3 fails to require operable linkage of the promoter to the fluorescent gene. The claim is also broad in that step (f) requires systemic fluorescence of any organ system and is not limited to the skeletal musculature affected by the $\alpha$ -actin promoter. Furthermore,

step (e) of claim 3 requires selection of eggs with fluorescence, which is not enabled as set forth below.

Claim 3 fails to require operable linkage between the promoter and the fluorescent gene. Placement of a promoter in a plasmid construct with a gene encoding a fluorescent product will not necessarily lead to expression of the gene. The claim should require operable linkage of the promoter to the gene. The specification teaches a use for a fish wherein the α-actin promoter drives expression of a reporter gene but does not teach how to use a fish wherein such expression fails to occur. Thus, the specification does not teach how to make the claimed fish expressing a fluorescent gene product wherein the promoter is not operably linked to the gene encoding a fluorescent gene product.

Claim 3 also requires selecting eggs with fluorescence. However, expression of a transgene will not occur in eggs because transcription does not occur until after the $10^{th}$ cleavage division at the midblastula transition (Zamir et al., **Developmental Dynamics**, 17:529-536, 1997). Therefore, fluorescence is not present in the fertilized egg as required by the claim. Accordingly, the specification does not teach fluorescence in fertilized eggs but teaches detecting fluorescence after 24 hours [paragraph 0024].

Finally, claim 3 requires cultivating eggs to produce golden zebrafish with systemic fluorescence. The α-actin promoter drives expression in skeletal muscle and does not drive expression in other organ systems. The term "systemic fluorescence" is so broad as to encompass fluorescence in systems other than skeletal muscle that are not supported or enabled by the specification. Accordingly, the claims should be limited to skeletal muscle expression.

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 3,4 and 6,7 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hsiao et al. [**Developmental Dynamics**, 220:323-336, April 2001] in view of Carvan et al [**Ann. N.Y. Acad. Sci.** 919:133-147, 2000].

Claims 3,4 and 6 are drawn to a method of making a transgenic zebrafish with systemic fluorescence of the skeletal musculature by injecting into a fertilized egg a linearized plasmid including flanking ITRs, an α-actin promoter that replaced a CMV promoter, and a fluorescent gene, selecting eggs with fluorescence and cultivating said eggs to produce zebrafish with systemic fluorescence. It is noted that claim 3 requires selection of fluorescent eggs, however, as set forth in the scope of enablement rejection above, the eggs would not be fluorescent due to the biology of zebrafish embryos because DNA is not transcribed until later in development. Therefore, for the purpose of considering the prior art, the step is read as selecting fluorescent embryos as is taught by the specification. Claim 4 limits the plasmid used to one of two specific plasmid constructs. Claim 6 limits the fluorescent gene to a green fluorescent gene. Claims 7 and 9 are drawn to the zebrafish made by the above methods.

Hsiao taught a method of making a transgenic zebrafish with systemic fluorescence of the skeletal musculature by injecting a linearized plasmid including flanking ITRs, an α-actin promoter that replaced a CMV promoter, a fluorescent EGFP gene and an SV40 polyA, into

fertilized zebrafish embryos and allowing fluorescent embryos to develop into zebrafish (Figure 1, second construct; page 325, col. 2, paragraph 2; page 333, col. 2, paragraphs 1 and 3). Hsiao taught using leopard strain zebrafish because they have less pigmentation than the AB strain (page 333, col. 2, paragraph 4). Lack of pigmentation is important in visualizing fluorescence (see page 323, col. 2, lines 1-2).

Hsiao et al did not teach using the golden strain of zebrafish as claimed.

However, the golden mutant strain of zebrafish was well known at the time of filing to produce less pigment as a result of a mutation at the *golden* locus. Carvan et al. taught the use of *golden* mutants to make transgenic zebrafish comprising transgenes encoding fluorescent gene products (page 141, paragraph 5). Carvan taught that golden mutants are preferred over other pigmentation mutants, such as *albino*, because *albino* mutants are poor breeders.

It would have been obvious to one of ordinary skill in the art at the time of filing to combine the technology taught by Hsiao of using ITR elements to enhance fluorescent reporter gene expression in transgenic zebrafish with the teachings of Carvan et al. in using golden mutant zebrafish. One of skill in the art would have been motivated to combine these teachings of Hsiao et al. with those of Carvan et al. to make the fluorescent transgene product more readily visible as visualization would not be obscured by pigment that arises in wild-type zebrafish beginning at day 3 of development.

One of skill in the art would have a reasonable expectation of success in combining the teachings of Hsiao et al. with those of Carvan et al. because the *golden* mutants are of the same species as the *leopard* mutants and differ only at the respective pigmentation loci.

It is noted that pα-actin-EGFP-ITR (8.1 kb) of Hsiao et al. (see page 333, col. 2, paragraph 1), absent evidence to the contrary, appears to be the same as pαEGFPITR (8.1 kb) of claim 4. Evidence that pα-actin-EGFP-ITR (8.1 kb) of Hsiao et al. is not the claimed pαEGFPITR (8.1 kb) may be sufficient to overcome the rejection as it relates to claim 4.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 5 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hsiao et al. [2001] in view of Carvan et al [2000] as applied to claims 3,4,6,7 and 9 above, and further in view of Finley et al, [**Biotechniques**, 31:66-72, July 2001].

As set forth above, Hsiao taught a method of making a transgenic zebrafish with systemic fluorescence of the skeletal musculature by injecting a linearized plasmid including flanking ITRs, an α-actin promoter that replaced a CMV promoter, a fluorescent EGFP gene and an SV40 polyA, into fertilized zebrafish embryos and allowing fluorescent embryos to develop into zebrafish. Carvan taught use of *golden* mutant zebrafish to facilitate visualization of fluorescent gene products. Neither Carvan nor Hsiao taught use of a fluorescent reporter gene encoding DsRed or the resultant fish exhibiting red fluorescence.

However, Finley et al. taught the use of several different fluorescent reporters in zebrafish, including DsRed. Finley also taught properties unique to DsRed such as low turnover and a unique emission spectra. Furthermore, Finley et al. taught that DsRed has a high signal to noise ratio, optimizing it as a reporter gene.

It would have been obvious to one of ordinary skill in the art at the time of filing to combine the technology taught by Hsiao and of Carvan et al. in making transgenic golden mutant zebrafish using ITR elements to enhance fluorescent reporter gene expression in transgenic zebrafish with the teachings of Finley et al. with respect to use of DsRed as a fluorescent reporter. One of skill in the art would have been motivated to combine these teachings of Hsiao et al. and Carvan et al. with those of Finley et al. because Finley et al taught advantages of DsRed over GFP as well as uses for multiple fluorescent reporter genes in the same fish.

One of skill in the art would have a reasonable expectation of success in combining the teachings of Hsiao et al and Carvan et al. with those of Finley et al. because the molecular techniques to make the claimed DsRed transgene were known and Finley taught transgene stability, expression and visualization.

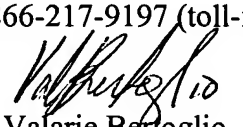
Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

***Conclusion***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Valarie Bertoglio whose telephone number is (571) 272-0725. The examiner can normally be reached on Mon-Thurs 5:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).



Valarie Bertoglio
Examiner
Art Unit 1632